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64 Integral multilayer analytical element.

67 An integral multilayer analytical element utilizable for determination of an analyte in a liquid sample such as a body fluid which comprises: a porous spreading layer of woven fabric or knitted fabric, which contains a hydrophilic cellulose derivative and a nonionic surfactant having an HLB value of not less than 10, a water-absorbing layer containing a hydrophilic polymer; and a light-transmissive, water-impermeable support. The spreading layer and/or water-absorbing layer may contain a reagent which participates in the detection reaction.

EP 0 162 301 A1

INTEGRAL MULTILAYER ANALYTICAL ELEMENTBACKGROUND OF THE INVENTIONField of the invention

This invention relates to a dry-type integral multi-
5 layer analytical element for determination of specific
component (i.e., analyte) in a liquid sample. More par-
ticularly, this invention relates to an integral multi-
layer analytical element advantageously employable in an
analysis of an aqueous liquid sample, especially in a
10 clinical test using a body fluid as a sample.

Description of prior arts

Until now, as dry-type analytical elements, there
have been proposed a number of integral multilayer analy-
tical elements (hereinafter occasionally referred to
15 simply as multilayer analytical elements) which comprise
a transparent support, a water-absorbing reagent layer
containing a hydrophilic polymer (binder) and a color-
forming reagent superposed on the support and a porous
spreading layer (hereinafter occasionally referred to
20 simply as spreading layer) as the uppermost layer. The
spreading layer of the multilayer analytical element func-
tions to spread therein an aqueous liquid sample (e.g.,
blood such as whole blood, plasma or serum, lymph,
saliva, spinal fluid, vaginal fluid, urine, drinking
25 water, liquors, river water, water discharged from fac-
tory, etc.) which has been applied onto the surface of
the spreading layer together with the components con-
tained in the liquid sample in a lateral direction to



European Patent
Office

EUROPEAN SEARCH REPORT

0162301

Application number

EP 85 10 4791

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	EP-A-0 103 901 (FUJI PHOTO FILM CO., LTD.) * Whole document *	1-4	G 01 N 33/52
X	EP-A-0 101 945 (FUJI PHOTO FILM CO., LTD.) * Whole document *	1-4	
X	EP-A-0 097 952 (FUJI PHOTO FILM CO. LTD.) * Whole document *	1-4	
X	EP-A-0 044 775 (EASTMAN KODAK CO.) * Whole document *	1-4	
X	GB-A-2 085 159 (FUJI PHOTO FILM CO. LTD.) * Whole document *	1-4	TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			G 01 N
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 23-07-1985	Examiner GRIFFITH G.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

EPO Form 1503, 03/82

4. An integral multilayer analytical element which comprises:

a porous spreading layer of woven fabric or knitted fabric, which contains a hydrophilic cellulose derivative
5 and a nonionic surfactant having an HLB value of not less than 10,

a water-absorbing or water-permeable reagent layer containing at least one reagent which is reactive to an analyte in the liquid sample to show a detectable change,

10 and

a light-transmissive, water-impermeable support.

CLAIMS:

1. An integral multilayer analytical element which comprises:
 - a porous spreading layer of woven fabric or knitted
5 fabric, which contains a hydrophilic cellulose derivative
and a nonionic surfactant having an HLB value of not less
than 10,
 - a water-absorbing layer containing a hydrophilic
polymer
10 and
 - a light-transmissive, water-impermeable support.
2. The integral multilayer analytical element as
claimed in claim 1, wherein said porous spreading layer
contains at least one reagent which is reactive to an
15 analyte in the liquid sample to show a detectable change.
3. The integral multilayer analytical element as
claimed in claim 2, wherein said absorbing layer contains
at least one reagent which is reactive to a reaction pro-
duct produced by a reaction between the analyte and the
20 reagent contained in the spreading layer so as to show a
detectable change.

Table 6

Glucose Concentration (mg/dl)	Measured Reflection Optical Density	
	Example 7	Com. Ex. 5
5		
103	0.640	0.271
252	1.005	0.360
320	1.142	0.410
450	1.353	0.460

Aqueous Liquid Sample	Spread Area (Measured Value)	
	Example 7	Com.Ex. 5
10 Human Serum	110 mm ²	270 mm ²
Versatol-P	115 mm ²	280 mm ²

The results given in Table 6 indicate that the integral multilayer analytical element having a knitted fabric spreading layer (which contains a hydrophilic cellulose derivative and a nonionic surfactant having an HLB of not less than 10) as prepared in the above Example 7 is superior to the integral multilayer analytical element having a woven fabric spreading layer (which contains a hydrophilic cellulose derivative and a nonionic surfactant having an HLB of not less than 10) as prepared in Example 6.

supply the liquid sample into the water-absorbing layer
(or a water-absorbing reagent-containing layer containing
a hydrophilic polymer) at a uniform volume per unit sur-
face area. This function is named a metering effect. As
5 the porous spreading layers, there have been proposed
non-fibrous porous spreading layers such as a non-fibrous
anisotropic microporous medium layer, typically a mem-
brane filter disclosed in Japanese Patent Provisional
Publication No. 49(1974)-53888 (Patent Publication No.
10 53(1978)-21677) and United States Patent No. 3,992,158,
three-dimensional lattice structure layer having conti-
nuous voids in which polymer microbeads are combined with
each other through an adhesive agent resistant to swell
with water disclosed in Japanese Patent Provisional Pub-
15 lication 55(1980)-90859 (United States Patent No.
4,258,001). These spreading layers are satisfactory in
the spreading action for a variety of of water. However,
if a whole blood sample is employed as the liquid sample,
the solid components such as red blood cell are apt to
20 plug the micro-voids of these spreading layers to disturb
the spreading action. Further, even in the case that a
plasma or serum containing a polymer component such as
protein at a high concentration level is employed as the
liquid sample, the polymer component is liable to plug
25 the micro-voids to disturb the spreading action. In con-
trast, a porous spreading layers of woven fabric dis-
closed in Japanese Patent Provisional Publications No.
55(1980)-164356 (United States Patent No. 4,292,272) and
No. 57(1982)-66359 can satisfactorily spread all of a
30 whole blood, a plasma, and a serum. Further, the spread-
ing layer of woven fabric is very advantageous from the
viewpoint of the easy handling for manufacture of an
integral multilayer analytical element, as well as from
the viewpoint of the superior mechanical strength of the
35 element.

It has been now found, however, that the spreading layer of woven fabric (hereinafter referred to as "woven fabric spreading layer") is hardly adjustable to vary the spread area (or spreadable area) from the inherently-
5 given spread area, maintaining the necessary metering effect. For instance, if an analyte content in an aqueous liquid sample (hereinafter occasionally referred to as simply "a liquid sample" or "test liquid") is small, it is desirable to decrease the spread area (i.e., area
10 in which the liquid sample is spread). In the case that the woven fabric spreading layer is employed as the spreading layer, the decrease of the spread area is difficultly attained or is only attained with reduce the metering action.

15

SUMMARY OF THE INVENTION

An object of the invention is to provide an integral multilayer analytical element having a porous spreading layer the spread area of which is easily adjustable over a wide range without reducing the spreading action
20 (metering action).

Another object of the invention is to provide an integral multilayer analytical element which is employable to quantitatively analyze a micro-amount of substance originating from a living body such as uric acid, creatinine, ammonia, and various enzymes (active value).
25

A further object of the invention is to provide an integral multilayer analytical element employing blood as the aqueous liquid sample the use of which facilitates determination of a relationship between a value measured
30 on a whole blood sample and a value measured on a plasma or serum sample and further facilitates conversion between the measured values.

A still further object of the invention is to pro-

vide an integral analytical element which makes it possible to determine a hematocrit value and a hemoglobin concentration in a whole blood sample.

The present invention provides an integral multi-
5 layer analytical element which comprises:

a porous spreading layer of woven fabric or knitted fabric, which contains a hydrophilic cellulose derivative and a nonionic surfactant having an HLB value of not less than 10,

10 a water-absorbing layer containing a hydrophilic polymer

and

a light-transmissive, water-impermeable support.

The present invention further provides an integral
15 multilayer analytical element which comprises:

a porous spreading layer of woven fabric or knitted fabric, which contains a hydrophilic cellulose derivative and a nonionic surfactant having an HLB value of not less than 10,

20 a water-absorbing or water-permeable reagent layer containing at least one reagent which is reactive to an analyte in the liquid sample to show a detectable change,

and

a light-transmissive, water-impermeable support.

25 Thus, the principal characteristic feature of the invention resides in the use of a spreading layer of woven fabric or knitted fabric and further in the incorporation of a hydrophilic cellulose derivative and a non-ionic surfactant having an HLB value of not less than 10.

30 DETAILED DESCRIPTION OF THE INVENTION

As the light-transmissive, water-permeable support of the integral multilayer analytical element of the invention, there can be mentioned light-transmissive,

water-impermeable supports employed in the multilayer analytical elements disclosed in the aforementioned patent publications. A representative support is a transparent polymer support having a thickness of approx. 50 μm to approx. 1 mm, preferably approx. 80 μm to approx. 300 μm . Examples of the polymer include polyethylene terephthalate, polycarbonate of bisphenol A, polystyrene, and cellulose esters (e.g., cellulose diacetate, cellulose triacetate, cellulose acetate propionate). If necessary, a subbing layer or an adhesive layer (which is known as such by the aforementioned patent publications) can be provided to the surface of support so as to strengthen the bonding between the support and the below-mentioned water-absorbing layer or reagent-containing layer.

The water-absorbing layer comprises as a main component a hydrophilic polymer which swells upon absorbing water, and accordingly is capable of absorbing a water reaching the surface thereof or permeating therein. The hydrophilic polymer can be chosen from natural or synthetic hydrophilic polymer having a swelling index of approx. 150 to 2,000 %, preferably approx. 250 to 1,500 % (water at 30°C). Examples of the hydrophilic polymer include gelatins (e.g., acid-treated gelatin, deionized gelatin, etc.), gelatin derivatives (e.g., phthalated gelatin, hydroxyacrylate-grafted gelatin, etc.), agarose, pulluran, pulluran derivatives, polyacrylamide, polyvinyl alcohol, and polyvinylpyrrolidone. The water-absorbing layer generally has a thickness of approx. 1 to 100 μm , preferably approx. 3 to 50 μm , most preferably approx. 5 to 30 μm , under dry condition. Preferably, the water-absorbing layer is transparent. The water-absorbing layer can contain a known mordant, basic polymer and/or acidic polymer.

The water-absorbing or water-permeable reagent-

containing layer (occasionally referred to simply as "reagent layer") is a substantially nonporous water-absorbing layer or a microporous water-permeable layer which contains a hydrophilic polymer (binder) and at least one reagent producing a detectable change upon reaction with an analyte (a component to be determined) in a liquid sample. The detectable change generally means a change which is detectable by a photometric measurement. Examples of the detectable change include color change, color formation, emission of fluorescence, change of absorbance in the ultraviolet region, and production of turbidity.

The reagent to be incorporated into the reagent layer is chosen according to the combination of an analyte in a liquid sample and a reaction system chosen for performing the analysis of the analyte. In the case that two or more reagents participate in the reaction, these reagents can be incorporated into one reagent layer, for instance, in the form of a mixture, and otherwise these reagents can be incorporated independently into plural layers. Examples of the reagent to be incorporated into the reagent layer include reagents containing enzyme(s) disclosed in the aforementioned patent publications, other known analytical reagent(s) and reagents employed for biochemical tests in clinical diagnosis.

The hydrophilic polymer employed for the formation of the substantially nonporous water-absorbing reagent layer serves as a medium for substantially uniformly dissolving or dispersing therein the reagent or reagent composition. Moreover, the hydrophilic polymer serves for absorbing the water of the liquid sample so as to supply the analyte together with water into the reagent layer. The hydrophilic polymer employed for this purpose can be selected from the water-absorbing hydrophilic polymers described hereinbefore with respect to the water-absorb-

ing layer. The substantially nonporous reagent layer generally has a thickness of approx. 3 to 50 μm , preferably approx. 5 to 30 μm , under dry condition. Preferably, the reagent layer is transparent.

5 The microporous water-permeable reagent layer is a microporous layer containing a reagent or a reagent composition within a microporous matrix layer formed by a solid particulate and a hydrophilic polymer (binder). In more detail, the microporous matrix layer is formed of
10 binding microporous particles or nonporous particles with the hydrophilic polymer binder to give a microporous continuous void structure.

Examples of the microporous particles and nonporous particles employed for the formation of the microporous
15 reagent layer include cellulose microparticles such as microcrystalline cellulose, cellulose micropowder, and cellulose micropartilces; silicon dioxide microparticles such as silica and diatomaceous earth; polymer beads, glass beads, and ceramic beads. The hydrophilic polymer
20 can be chosen from the water-absorbing hydrophilic polymer described hereinbefore with respect to the water-absorbing layer. Also employable is an aqueous latex of a copolymer containing a hydrophilic group as a repeating constitutional unit in an amount of not less than approx.
25 2 %, as disclosed in Japanese Patent Provisional Publication No. 59(1984)-145965. The microporous reagent layer generally has a thickness of approx. 7 to 50 μm , preferably approx. 10 to 30 μm , under dry condition.

The reagent layer can contain a known pH buffer composition, a polymer pH buffer, a basic polymer, an acidic polymer, a polymer mordant, etc.

The water-absorbing layer or the substantially nonporous reagent layer can be formed on the aforementioned light-transmissive, water-impermeable support according
35 to a known coating method. The microporous reagent layer

can be formed on the support according to the method disclosed in Japanese Patent Application No. 57(1982)-227980 or Japanese Patent Provisional Publication No. 59(1984)-145965. If necessary, the surface of the support can be
5 processed according to the known physico-chemical or chemical activation method, or provided with a transparent subbing layer (e.g., gelatin subbing layer), so that the bonding between the support and the water-absorbing layer or between the support and the reagent layer can be
10 strengthened. Moreover, an water-absorbing layer can be provided between the support and the reagent layer. Particularly, the provision of a water-absorbing layer between the support and the reagent layer is preferred in the case that the reagent layer is a microporous reagent
15 layer.

On the reagent layer or water-absorbing layer, a light-shielding layer can be formed. The light-shielding layer is a water-passable or water-permeable layer in which light-shielding microparticles or a light-shielding
20 and light-reflective microparticles is dispersed in a small amount of a film-forming hydrophilic polymer. The light-shielding layer shields a color of an aqueous liquid sample, particularly the red color of hemoglobin contained in a whole blood sample, applied to the below-
25 described porous spreading layer in the reflection measurement of the detectable change taking place in the reagent layer or water-absorbing layer on the light-transmissive support side. Further, the light-shielding layer can function as a light-reflecting layer and a
30 background layer.

Examples of the light-shielding and light-reflecting particles include titanium dioxide microparticles (e.g., titanium dioxide microcrystalline particles of the rutile type, anatase type or brookite type, having particle size
35 of approx. 0.1 to 1.2 μm), barium sulfate microparticles,

and aluminum microparticles or micro-flakes. Examples of the light-shielding microparticles include carbon black, gas black and carbon microbeads. Among these microparticles, titanium dioxide microparticles and barium sulfate microparticles are preferred. As the film-forming hydrophilic polymer, there can be mentioned the hydrophilic polymer stated hereinbefore with respect to the water-absorbing layer, and weak water-hydrophilic regenerated cellulose and cellulose acetate. Among these polymers, gelatin, gelatin derivatives, and polyacrylamide are preferred. The gelatin and gelatin derivatives can be employed in combination with a known hardening agent (i.e., cross-linking agent).

The light-shielding layer can be formed on the reagent layer or water-absorbing layer by coating an aqueous dispersion containing light-shielding microparticles and a hydrophilic polymer and then drying the coated layer in a known manner. The volume ratio of the hydrophilic polymer to the light-shielding microparticles under dry condition ranges generally from approx. 2.5 to 7.5, preferably from approx. 3.0 to 6.5, to 10 of the microparticles. In the case that the light-shielding microparticles are titanium dioxide microparticles, the hydrophilic polymer is incorporated in a weight ratio of generally approx. 0.6 to 1.8, preferably approx. 0.8 to 1.5, to 10 of the titanium dioxide. The light-shielding layer has a thickness of approx. 3 to 30 μm , preferably approx. 5 to 20 μm , under dry condition.

On the reagent layer, water-absorbing layer or light-shielding layer, an adhesive layer can be provided to assist the provision of the below-mentioned porous spreading layer. Especially in the case that the light-shielding layer is arranged, an adhesive layer is preferably incorporated. The adhesive layer can be composed mainly of a hydrophilic polymer which can be united with

the porous spreading layer under such conditions that the adhesive layer is wetted or swollen with water. As the hydrophilic polymer, there can be mentioned the hydrophilic polymers described hereinbefore with respect to the water-absorbing layer. Among these hydrophilic polymers, gelatin, gelatin derivatives and polyacrylamide are preferred. The adhesive layer has a thickness of approx. 0.5 to 20 μm , preferably approx. 1 to 10 μm , under dry condition. The adhesive layer can contain a surfactant. The surfactant preferably is a nonionic surfactant, especially a nonionic surfactant having a chain structure of 8 to 15 oxyethylene or oxypropylene groups.

The adhesive layer can be formed on the reagent layer, water-absorbing layer or light-shielding layer by coating an aqueous solution containing a hydrophilic polymer and an optionally added surfactant in a known manner.

On the water-absorbing layer or reagent layer is a provided a porous spreading layer of woven fabric (or woven fabric-like material) or knitted fabric (or knitted fabric-like material) directly or via a light-shielding layer (or light-reflecting layer) and an adhesive layer. The porous spreading layer is capable of spreading laterally therein an aqueous liquid sample which has been applied onto the upper surface thereof (surface on the side farthest from the light-transmissive support) to supply the components of the liquid sample into the water-absorbing layer or water-absorbing or water-permeable reagent layer (containing a hydrophilic polymer) at uniform volume per unit surface area, that is, a spreading action or metering action. The porous spreading layer also functions as a filter layer to remove insoluble material in an aqueous liquid sample (e.g., red blood cell in a whole blood sample) which is apt to disturb the analysis.

As the woven fabric (including woven fabric-like material), there can be mentioned a variety of woven fabric disclosed in Japanese Patent Provisional Publications No. 55(1980)-164356 and No. 57(1982)-66359. Among the woven
5 fabrics, a plain weave cloth fabricated with warp and weft is preferred, and particularly preferred are sheeting cloth, shirting cloth, broad cloth and popline cloth. The yarns can be of any material mentioned hereinafter with respect to the knitted fabric. The yarn can be a
10 filament yarn or spun yarn. The spun yarn is preferred. The yarn of the woven fabric generally is in the range of approx. 20 S to 150 S (in terms of cotton spun yarn count), preferably approx. 40 S to 120 S, or in the range of approx. 35 D to 300 D (in terms of silk yarn denier),
15 preferably approx. 35 D to 90 D. The thickness of the woven fabric ranges generally from approx. 100 μ m to approx. 500 μ m, preferably approx. 120 μ m to approx. 350 μ m. The void ratio of the woven fabric ranges generally from approx. 40 % to approx. 90 %, preferably from
20 approx. 50 % to approx. 85 %.

As the knitted fabric (including knitted fabric-like material), there can be mentioned a wide variety of knitted materials such as warp knitting and weft knitting. Examples of the warp knitting include single atlas stitch
25 fabric, single tricot stitch fabric, double tricot stitch fabric, milanese stitch fabric and raschel stitch fabric. Examples of the weft knitting include plain stitch fabric, pearl stitch fabric, rib stitch fabric and interlock stitch fabric. The knitted fabric is generally made of
30 natural yarns such as cotton yarn, silk yarn and wool yarn; and yarns (including filaments) of regenerated cellulose such as viscous rayon and cuprammonium rayon, semisynthetic organic polymers such as cellulose diacetate and cellulose triacetate, and synthetic organic
35 polymers such as polyamide (e.g., various nylon), acetal-

lized polyvinyl alcohol (e.g., vinylon), polyacrylnitrile, polyethylene terephthalate, polyethylene, polypropylene and polyurethane, and mixtures of natural yarns and regenerated yarns, semisynthethic organic polymer
5 yarns and synthetic organic polymer yarns. The yarn can be a filament yarn or spun yarn. The spun yarn is preferred. The yarn of the knitted fabric generally is in the range of approx. 40 S to 150 S (in terms of cotton spun yarn count), preferably approx. 60 S to 120 S, or in
10 the range of approx. 35 D to 130 D (in terms of silk yarn denier), preferably approx. 35 D to 90 D. The gauge number adopted for manufacturing the knitted fabric generally ranges from approx. 20 to approx. 50. The thickness of the knitted fabric ranges generally from approx. 100
15 μ m to approx. 600 μ m, preferably approx. 150 μ m to approx. 400 μ m. The void ratio of the knitted fabric ranges generally from approx. 40 % to approx. 90 %, preferably from approx. 50 % to approx. 85 %. Among the warp knittings, single tricot stitch fabric, double tri-
20 cot stitch fabric, milanese stitch fabric and raschel stitch fabric are preferred, because these are hardly stretchable in the warp direction, are easily handled in the below-described lamination process of the knitted fabric spreading layer, and hardly become unlaced.

25 The woven fabric or knitted fabric to be employed for the formation of the knitted fabric spreading layer is substantially free from oil and fat which is apt to attach or supplied to the fabric in the course of the yarn manufacture or fabric manufacture and removed by a
30 defatting (deoiling) process such as washing with water. The woven fabric or knitted fabric can be made hydrophilic, for instance, by a physical activation treatment (preferably, glow-discharge treatment or corona-discharge treatment) applied to at least one side of the woven
35 fabric or knitted fabric as disclosed in Japanese Patent

Provisional Publication No. 57(1982)-66359; or by a surfactant impregnation treatment (preferably, a nonionic surfactant impregnation treatment), or a hydrophilic polymer impregnation treatment as disclosed in Japanese Patent Provisional Publications No. 55(1980)-164356 and No. 57(1982)-66359; or an optional combination of two or more these treatments. Thus treated hydrophilic woven fabric or knitted fabric is satisfactory in the adhesion to the underlayer.

- 10 The woven fabric or knitted fabric can be laminated under adhesion on the reagent layer, water-absorbing layer or adhesive layer in the manner as disclosed in Japanese Patent Provisional Publications No. 55(1980)-164356 and No. 57(1982)-66359, etc. In more detail, a
15 woven fabric or knitted fabric is applied under substantially uniform light pressure to the undried reagent layer, water-absorbing layer or adhesive layer which is provided just after the coating procedure is complete. Alternatively, the dried reagent layer, water-absorbing
20 layer or adhesive layer is swollen by supplying water substantially uniformly into the layer, and the woven fabric or knitted fabric is then applied onto the swollen layer in the same manner as above. Thus, the woven fabric or knitted fabric is combined with other layers to
25 give the integral structure. In the case of employing gelatin or a gelatin derivative as the hydrophilic polymer binder in the reagent layer, water-absorbing layer or adhesive layer, the woven fabric or knitted fabric can be applied onto the undried gelatin (or gelatin derivative)
30 layer still under a gelation condition just after the coating is complete, so as to give the desired integral structure.

 Examples of the hydrophilic cellulose derivative incorporatable into the porous spreading layer of the
35 integral multilayer analytical element of the present

invention include cellulose ethers a portion or whole of the hydroxyl groups of which are substituted with lower alkyl groups containing 1 - 3 carbon atoms or hydroxyl-substituted lower alkyl groups containing 1 - 4 carbon atoms. Among these cellulose ethers, water-soluble cellulose ethers are preferred. Examples of the cellulose ethers include methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose, and hydroxybutylmethylcellulose. The hydrophilic cellulose derivative is generally incorporated into the porous spreading layer in the amount of approx. 0.5 to 15 g. (preferably approx. 0.7 to 10 g.) per 1 m² of the porous spreading layer.

As the nonionic surfactant having an HLB value (Hydrophile-Lipophile-Balance defined in J. Soc. Cosmet. Chem., 1, 311(1949) and "Kagaku(Science)" 23, 546(1953)) of not less than 10, there can be mentioned ethyleneoxide addition compounds of polyhydric alcohol esters (condensates), polyethylene glycol monoesters, polyethylene glycol diesters, ethyleneoxide addition compounds of higher alcohols (condensates), ethyleneoxide addition compounds of alkylphenols (condensates), and alkanolamides of higher fatty acids. The nonionic surfactants having an HLB value of not less than 10 can be employed in combination. Moreover, the nonionic surfactants having an HLB value of not less than 10 can be employed in combination with other surfactants such as a nonionic surfactant having an HLB value of less than 10. In the latter case, the HLB value can be suitably adjusted.

Examples of the nonionic surfactant having an HLB value of not less than 10 are given below together with HLB values thereof. It should be understood that the nonionic surfactant of the invention is not restricted to the below-listed nonionic surfactants.

	<u>Nonionic Surfactant</u>	<u>HLB Value</u>
	POE (20) Sorbitan monooleate	15.0
	POE (10) Sorbitan monooleate	13.5
	POE (4) Sorbitan tristearate	10.5
5	POE (4) Trioleate	11.0
	POE (30) Stearate	16.0
	POE (40) Stearate	16.9
	POE (100) Stearate	18.8
	PEG (400) Monostearate	11.6
10	PEG (400) Monolaurate	13.1
	PEG (1000) Dilaurate	14.1
	PEG (1540) Distearate	14.8
	EO (6) Condensate of lauryl alcohol	11.8
	EO (10) Condensate of lauryl alcohol	14.1
15	EO (30) Condensate of lauryl alcohol	17.4
	EO (20) Condensate of oleyl alcohol	15.3
	EO (20) Condensate of cetyl alcohol	15.7
	POE (10) Octylphenyl ether	13.5
	POE (15) Octylphenyl ether	15.1
20	POE (30) Octylphenyl ether	17.4
	POE (12) Nonylphenyl ether	14.1
	POE (20) Nonylphenyl ether	16.0
	Triethanolamine oleate	12.0
Remark)	POE: polyethyleneoxide	
25	PEG: polyethylene glycol	
	EO: ethylene oxide	

Numeral put in parentheses means a number of condensed ethyleneoxide units.

The nonionic surfactant is incorporated into the porous spreading layer generally in the range of approx. 0.1 to 3 g., preferably approx. 0.2 to 2 g., per 1 m².

The hydrophilic cellulose derivative and nonionic surfactant having an HLB value of not less than 10 can be incorporated into the porous spreading layer in any of

the manners described below. Both are mixed to give an aqueous solution, and the solution is substantially uniformly coated or sprayed over the porous spreading layer and dried. Alternatively, a woven fabric or knitted
5 fabric is dipped into the solution containing both components and then laminated under dry or semidry condition on the water-absorbing layer, reagent layer or adhesive layer to give a composite structure. The hydrophilic cellulose derivative is initially introduced into the
10 porous spreading layer in the above-mentioned manner, and then the nonionic surfactant in an organic solvent (e.g., acetone, etc.) is introduced into the spreading layer in the same manner. The order of the introduction of the components stated above can be reversed. Further, both
15 components can be introduced into the porous spreading layer simultaneously with introduction of other reagents.

The reagent composition or a portion or component thereof can be incorporated into the porous spreading layer. Especially in the case that the reagent composition includes a polymer substance, typically enzyme, or a
20 dissociating agent for analyte conjugated with protein, said reagent component is almost preferably incorporated into the porous spreading layer. The incorporation of the reagent composition or a portion or component thereof
25 into the spreading layer can be done in the manner as disclosed in Research Disclosure #12626 (October 1974), Japanese Patent Provisional Publication No. 50(1975)-137192 (corresponding to United States Patent No. 3,983,002) and Japanese Patent Provisional Publication
30 No. 57(1982)-208997 (corresponding to United States Patent No. 4,452,887), etc. for enzyme; Japanese Patent Provisional Publication No. 57(1982)-208998, etc. for substrate of enzyme; Japanese Patent Provisional Publication No. 59(1984)-171864, etc. for a dissociating
35 agent for analyte associated with protein; Japanese

Patent Provisional Publication No. 59(1984)-49854
(Example 7), etc. for other components.

The integral multilayer analytical element of the present invention can be provided, if necessary, with a
5 detection layer or a mordant layer as disclosed in Japanese Patent Provisional Publication No. 51(1976)-40191
(corresponding to United States Patent No. 4,042,335), Japanese Patent Provisional Publication No. 53(1978)-
131089 (corresponding to United States Patent No.
10 4,144,306), etc.; a migration-inhibition layer as disclosed in Japanese Patent Provisional Publication No.
54(1979)-29700 (corresponding to United States Patent No. 4,166,093), etc.; an intermediate layer as disclosed in
Japanese Patent Provisional Publication No. 51(1976)-
15 40191; a reagent-containing microparticle-dispersing layer as disclosed in Japanese Patent Provisional Publication No. 56(1981)-8549 (corresponding to United States
Patent No. 4,356,149); a uniform thin barrier layer of a specific hydrophobic polymer as disclosed in Japanese
20 Patent Provisional Publication No. 52(1977)-3488 (corresponding to United States Patent (Re) No. 30,267); an air
barrier layer disclosed in Japanese Patent Provisional Publication No. 58(1983)-77661 and Japanese Patent Application No. 58(1983)-153822; and a gas-permeable adhesive
25 intermediate layer as disclosed in Japanese Patent Application No. 58(1983)-128759.

The integral multilayer analytical element of the present invention can be cut to give a small square chip
(length of one side: approx. 15 to 30 mm) or round sheet
30 of almost same size and encased in a slide frame, etc. to give an analytical slide as disclosed in Japanese Patent
Provisional Publication No. 57(1982)-63452, Japanese Patent Provisional Publication No. 54(1979)-156079 (corresponding to United States Patent No. 4,169,751), Japanese
35 Utility Model Provisional Publication No. 56(1981)-142454

(corresponding to United States Patent No. 4,387,990), Japanese Utility Model Provisional Publication No. 58(1983)-32350, and Japanese Patent Provisional PCT Publication No. 58(1983)-501144 (corresponding to WO 83/00391). The analytical element in the form of the analytical slide is advantageous in all aspects such as manufacture, packing, transfer, storage and measurement procedure.

The integral multilayer analytical element of the invention can be used in the manner as disclosed in the aforementioned patent publications. For instance, an aqueous liquid sample in the amount of approx. 5 to 30 μ l, preferably approx. 8 to 15 μ l, is spotted on the porous spreading layer, and if necessary (particularly in the case of employing an enzyme as a component of the reagent composition) the analytical element is then subjected to incubation at a substantially constant temperature in the range of approx. 20 to 45°C. The element is photometrically measured through reflection photometry on the light-transmissive support side to detect a detectable change such as color change or color formation shown in the element. The measured value was colorimetrically treated to quantitatively determine the analyte in the liquid sample. In the case of using an integral multilayer analytical element having a water-absorbing layer and a woven fabric or knitted fabric spreading layer on a light-transmissive, water-impermeable support, a hematocrit value or hemoglobin concentration in a blood can be determined in the same manner as in the case of using an integral multilayer analytical element as disclosed in Japanese Patent Provisional Publication No. 56(1981)-96245 (United States Patent No. 4,340,565) and Japanese Patent Provisional Publication No. 56(1981)-97872 (United States Patent No. 4,337,222).

The integral multilayer analytical element of the

present invention can be prepared in the form of having a woven fabric or knitted fabric porous layer for supplying an aqueous liquid sample via a fixed area defined by a certain shape thereof (i.e., patch) which is provided as the uppermost layer, as disclosed in Japanese Utility Model Provisional Publication No. 57(1982)-42951.

The present invention is further illustrated by the following examples and comparison examples.

Example 1

10 An aqueous gelatin (alkali-treated and deionized) solution was coated on a gelatin subbing layer of a colorless, transparent polyethylene terephthalate (PET) film (support, thickness: 180 μm) to give a layer of thickness of 20 μm (dry basis). The coated layer was
15 then dried to give a water-absorbing layer.

One surface of a plain weave broad cloth (thickness: 140 μm) of polyethylene terephthalate (PET) spun yarn (count: equivalent to approx. cotton count 100 S) was processed by glow discharge for 60 sec. (1.6 kW/m^2 , oxygen concentration was controlled under reduced pressure).
20 The processed cloth was then treated with an acetone solution containing 1 wt% of hydroxypropylcellulose (methoxy group content: 28 - 30 %, hydroxypropoxy group content: 7 - 12 %, viscosity: 50 cps at 20°C in 2% aqueous solution) and 1 wt% of polyoxyethylene octylphenyl ether (number of added ethylene oxides : 30 moles, HLB value: 17.4). Thus, the cloth was impregnated with the nonionic surfactant and hydrophilic cellulose derivative.

On the water-absorbing layer having been almost uniformly wetted with water was placed the treated broad cloth to face the glow discharge-processed surface with the water-absorbing layer. The composite was then passed between pressure rollers to uniformly combine the plain

weave broad cloth with the water-absorbing layer. Thus, an integral multilayer analytical element having a woven fabric spreading layer was prepared.

Comparison Examples 1 & 2

5 The procedures of Example 1 were repeated except that the plain weave broad cloth was impregnated with the nonionic surfactant only (Comparison Example 1) or with the hydrophilic cellulose derivative only (Comparison Example 2). Thus, two integral multilayer analytical
10 elements were produced. The spread area was measured in the same manner as in Example 1. The results are set forth in Table 1.

On the woven fabric spreading layer of thus produced integral multilayer analytical element (prepared in Exam-
15 ple 1 or Comparison Example 1 or 2) was spotted 10 μ l of human plasma. The period of time for spreading and the spread area were measured. The results are set forth in Table 1.

Table 1

20 Analytical Element	Spread Area	Period for Spreading	Condition of Spreading
Example 1	120 mm ²	10 sec.	Uniform
Com. Ex. 1	250 mm ²	10 sec.	Uniform
Com. Ex. 2	150 mm ²	25 sec.	Non-uniform

25 The integral multilayer analytical element of the invention was favorable in the spread area, period of

time needed for the completion of the spreading action and condition of the spreading action. In contrast, the multilayer analytical element of Comparison Example 1 (in which the woven fabric spreading layer was impregnated with only a nonionic surfactant of an HLB value 17.4) showed an excessively large spread area approx. twice as large as a preferred spread area, while the multilayer analytical element of Comparison Example 2 (in which the woven fabric spreading layer was impregnated with only a hydrophilic cellulose derivative) showed a unsuitable spreading condition.

Example 2

The procedures of EXample 1 were repeated except that the composition of the solution for treating the plain weave broad cloth was replaced with the following composition.

Plain Weave Broad Cloth Treating Solution

Methylcellulose (viscosity 50 cps at 20°C, measured on 2 % aqueous solution)	2 g.
20 Polyoxyethylene octylphenyl ether (containing 15(average) oxyethylene units, HLB: 15.1)	1 g.
Water	98 g.

Thus, an integral multilayer analytical element was prepared.

25 On the woven fabric spreading layer of the multilayer analytical element was spotted a human plasma in an amount of from 2 μ l to 12 μ l. A relationship between the spotted amount (x μ l) and the spread area (x mm²) is expressed by the following linear equation:

$$30 \quad y = 15.67x + 1.81.$$

The correlation coefficient (r) is 0.9995.

Example 3

The procedures of Example 1 were repeated except that the composition of the solution for treating the 5 plain weave broad cloth was replaced with the following composition using surfactants having different HLB values.

Plain Weave Broad Cloth Treating Solution

Methylcellulose (viscosity 50 cps at 20°C,		
10	measured on 2 % aqueous solution)	2.5 g.
	Nonionic surfactant (indicated in Table 2)	1 g.
	Water	98 g.

Thus, an integral multilayer analytical element was prepared.

- 15 On the woven fabric spreading layer of the multilayer analytical element was spotted 7% aqueous albumin solution in the amount of from 10 μ l. The spread area was measured. The results are set forth in Table 2.

Table 2

HLB Value	Nonionic Surfactant	Spread Area
5	4.5 PrG monolaurate	140 mm ²
	6.1 DiEG monostearate	125 mm ²
	7.8 PEG(200) stearate monoester	115 mm ²
	10.5 POE(4)Srb tristearate	85 mm ²
	11.8 EO(6) condensate of lauryl alcohol	67 mm ²
10	15.1 POE(15) OctPh ether	43 mm ²
	16.0 POE(20) NonPh ether	60 mm ²
	17.0 (*) POE(30) OctPh ether 82.6 %	
	POE(15) OctPh ether 17.4 %	50 mm ²
15	17.5 (*) POE(100) stearate 54 %	
	POE(30) OctPh ether 46 %	42 mm ²

Remarks PrG: propylene glycol, EG: ethylene glycol,
Srb: sorbitan, OctPh: octylphenyl, NonPh: nonyl-
phenyl

20 Other expressions are the same as in the
aforementioned list of the nonionic surfactant.

(*): The HLB value was adjusted using the two
nonionic surfactants having different HLB values in
the indicated ratio.

25 It is apparent from the results given in Table 2
that the spread area of the aqueous albumin solution
(aqueous liquid sample) was well controlled to give a
very small area in the use of the integral multilayer
analytical element of the invention having a woven fabric
spreading layer containing a nonionic surfactant of an

HLB value of not less than 10 and a hydrophilic cellulose derivative.

Example 4 & Comparison Example 4

An aqueous gelatin (alkali-treated and deionized) solution was coated on a gelatin subbing layer of a colorless, transparent PET film (support, thickness: 180 μm) to give a layer of 20 μm thick (dry basis). The coated layer was dried to give a water-absorbing layer.

One surface of a plain weave broad cloth (thickness: 140 μm) of polyethylene terephthalate (PET) spun yarn (count: equivalent to approx. cotton count 100 S) was processed by glow discharge for 60 sec. (1.6 kW/m^2 , oxygen concentration was controlled under reduced pressure).

On the water-absorbing layer having been almost uniformly wetted with water was placed the processed broad cloth to face the glow discharge-processed surface with the water-absorbing layer. The composite was then passed between pressure rollers to uniformly combine the plain weave broad cloth with the water-absorbing layer.

On the laminated woven fabric spreading layer was coated the aqueous solution for treating the spreading layer having the following composition in the amount to give the coated methylcellulose amount indicated in Table 3 per 1 m^2 .

Plain Weave Broad Cloth Treating Solution

Methylcellulose (viscosity 50 cps at 20°C , measured on 2 % aqueous solution)	20 g.
Titanium dioxide microparticles (rutile type, particle size 0.25 - 0.40 μm)	100 g.
Polyoxyethylene octylphenyl ether (containing 30(average) oxyethylene units, HLB: 17.4)	10 g.
Water	1000 g.

The coated solution was dried to prepare an integral multilayer analytical element (Example 3, three kinds of elements were prepared).

An integral multilayer analytical element was prepared in the same manner as in Example 4 except that the woven fabric layer was not treated with the plain weave broad cloth treating solution (Example 4).

On each of the woven fabric spreading layer of the multilayer analytical elements (Example 4 and Comparison Example 3) was spotted 10 μ l of a human plasma, and the element was subjected to incubation at 37°C for 6 min. The spread area was then measured. Further, the background optical density was measured through the support by reflection photometry. This optical density indicates the light-shielding or light-reflecting function (white background function) of the titanium dioxide microparticles dispersed in the hydrophilic cellulose derivative in the woven fabric spreading layer. The measured values are set forth in Table 3.

20

Table 3

Methylcellulose Coated Amount	Spread Area	Reflection Optical Density
0.95 g/m ²	110 mm ²	0.21
1.9	105	0.25
25 9.5	102	0.25
Not-coated(Com.Ex.3)	155	0.54

The results given in Table 3 indicate that the

spread area of the aqueous liquid sample in the woven fabric spreading layer of the multilayer analytical element of the invention is small over a wide hydrophilic cellulose derivative concentration range and further that
5 the background optical density is also small in the range. In contrast, the woven fabric spreading layer containing a large amount of a nonionic surfactant having an HLB value of not less than 10 but containing no hydrophilic cellulose derivative (multilayer analytical element of
10 Comparison Example 3) gives a large spread area in excess of approx. 40 to 60 % from a preferred value and further shows practically disadvantageous background optical density.

Example 5 & Comparison Example 4

15 The procedures of Example 1 were repeated except that the spreading layer was prepared from a plain weave broad cloth (thickness: 180 μ m) of cotton spun yarn (cotton count: 80 S, no glow-discharge treatment had been applied) or a plain weave broad cloth (thickness: 180 μ m)
20 of PET/cotton(75%/25%) mixed spun yarn (count: equivalent to cotton count 80 S, glow-discharge treatment had been applied). Thus, two integral multilayer analytical elements having different woven fabric spreading layers were prepared (Example 5).

25 The procedures of Example 5 were repeated except that the woven fabric spreading layer was not impregnated with the combination of a nonionic surfactant of an HLB value of not less than 10 and a hydrophilic cellulose derivative. Thus, two integral multilayer analytical
30 elements having different woven fabric spreading layers were prepared (Comparison Example 5).

On each of the woven fabric spreading layers of the four multilayer analytical elements was spotted 10 μ l of

a human plasma. The spread area and the spreading conditions were then determined. The results are set forth in Table 4.

Table 4

5 Woven Fabric Spreading Layer		Spread Area	Spreading Conditions
10	Cotton Broad Example 5	83 mm ²	Uniform
	Com. Ex. 4	131 mm ²	Uniform
	PET/Cotton Example 5	90 mm ²	Uniform
	Com. Ex. 4	155 mm ²	Less Uniform

The results given in Table 4 indicate that the multilayer analytical element of the present invention gives uniform spreading to the spotted aqueous liquid sample and shows small spreading area regardless of the material of the porous spreading layer, namely, cotton broad cloth or PET/cotton mixed broad cloth. In the multilayer analytical element of Comparison Example 4, the spreading conditions and spread area are almost the same as those given by the multilayer analytical element of the invention in the case of using a PET/cotton broad cloth as the porous spreading layer material. However, in the case of using a cotton broad cloth as the spreading layer material, the spreading conditions are less uniform, and the spread area exceeds a preferred value by approx. 30 to 50 %.

Example 6 & Comparison Example 5

A reagent layer for glucose determination (thickness: approx. 15 μm , dry basis) was formed on a gelatin subbing layer of a colorless, transparent PET film support (thickness: 185 μm) using the following composition.

Reagent Composition for Glucose Determination

Glucose oxidase	15,000 IU
Peroxidase	25,000 IU
1,7-Dihydroxynaphthalene	5 g.
10 4-Aminoantipyrine	12 g.
Deionized gelatin	200 g.
Polyoxyethylene nonylphenyl ether (containing 10(average) oxyethylene units)	2 g.

On the reagent layer was coated an aqueous dispersion containing 0.2 g. of polyoxyethylene nonylphenyl ether (containing 10(average) oxyethylene units), 8 g. of titanium dioxide microparticles (rutile type, particle size: 0.25 - 0.40 μm), and 1 g. of deionized gelatin. The coated layer was dried to form a light-shielding layer of approx. 5 μm thick (dry basis).

On the light-shielding layer was coated a solution of 4 g. of deionized gelatin and 0.2 g. of polyoxyethylene nonylphenyl ether (containing 10(average) oxyethylene units) in 100 ml of water. The coated layer was dried to form an adhesive layer of approx. 2 μm thick (dry basis).

One surface of a plain weave broad cloth (thickness: 140 μm) of PET spun yarn (count: equivalent to cotton count 100 S) was processed by glow discharge for 60 sec. (1.6 kW/m^2 , oxygen concentration was controlled under reduced pressure).

On the adhesive layer having been almost uniformly wetted with water was placed the processed broad cloth to

face the glow discharge-processed surface with the adhesive layer. The composite was then passed between pressure rollers to uniformly combine the plain weave broad cloth with the adhesive. Thus, a woven fabric spreading 5 layer was formed.

On the spreading layer was coated the woven fabric spreading layer treating solution having the below-stated composition in the amount of 150 ml per 1 m². The coated layer was dried to give an integral multilayer analytical 10 element for quantitative determination of glucose concentration in blood (Example 6).

Woven Fabric Spreading Layer Treating Solution

Methylcellulose (viscosity 50 cps at 20°C, measured on 2 % aqueous solution)	20 g.
15 Titanium dioxide microparticles (rutile type, particle size: 0.25 - 0.40 μm)	100 g.
Polyoxyethylene octylphenyl ether (containing 30(average) oxyethylene units, HLB: 17.4)	10 g.
Water	1000 g.

20 Separately, an integral multilayer analytical element for quantitative determination of glucose concentration in blood was prepared in the same manner as above (Example 6) except that the porous spreading layer was not treated with the woven fabric spreading layer 25 treating solution (Comparison Example 5).

On each of the integral multilayer analytical elements for quantitative determination of glucose in blood (the elements of Example 6 and Comparison Example 5) was spotted 10 μl of a human serum having the glucose content 30 indicated in Table 3 which was prepared by adding glucose to a human serum. The element was subjected to incubation at 37°C for 6 min. The color formation shown on the element was then measured by reflection photometry with a

visible ray of 500 nm (central wavelength) using a reflection optical densitometer. The measured values are set forth in Table 5.

Table 5

5 Glucose Concentration (mg/dl)	<u>Measured Reflection Optical Density</u>	
	Example 6	Com. Ex. 5
103	0.497	0.271
252	0.750	0.360
320	0.839	0.410
10 450	0.982	0.460

Aqueous Liquid Sample	<u>Spread Area (Measured Value)</u>	
	Example 6	Com.Ex. 5
Human Serum	128 mm ²	270 mm ²
Versatol-P	127 mm ²	280 mm ²

15 Remark: The glucose concentration value of the human serum was obtained by Hexokinase-G-6-PDH method. Versatol-P; control serum, tradename of Werner-Lambert Corp.

The results set forth in Table 5 indicate that the
 20 integral multilayer analytical element of the invention gives a calibration curve having an incline larger than that given by the integral multilayer analytical element of the Comparison Example. Further, the integral multilayer analytical element of the invention gives a calib-

ration curve having a large incline even in the region of high glucose concentration. For the reasons, the analytical element of the invention makes it possible to determine very accurately the glucose content over wide 5 range.

Example 7

One surface of a tricot knitted fabric (thickness: approx. 250 μm) of PET spun yarn (equivalent to approx. 50 D) having been defatted by washing with water was processed by glow discharge for 60 sec. (1.6 kW/m^2 , oxygen concentration was controlled under reduced pressure). 10

The procedures of Example 6 were repeated except that the spreading layer was prepared from the above-processed knitted fabric. Thus, an integral multilayer 15 analytical element for quantitative determination of glucose.

This element was subjected to the same measurement as in Example 6. The results are set forth in Table 6.